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"Nanoscale Monitoring and Modulation of Auditory Sensing System"

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14. ABSTRACT
This is the final report of a project to understand the biophysical mechanisms underlying hair cell activity in the auditory system.

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I. Abstract

The auditory system can detect induced deflections as small as a few Å, while being able to withstand applied pressures spanning orders of magnitude. However, the biophysical mechanisms underlying the hair cell activity have still not been fully explained, making the auditory system among the least understood of the biological sensory systems. Here, with the collaboration of Prof. D. Bozovic (Dept. of Physics, UCLA) we developed a different technique to impose steady-state deflections on un-loaded hair cells. A magnetic bead (1 μm in diameter) was attached to the stereocilia of a selected bundle, and a magnetic probe brought to its vicinity. This enabled us to apply a force on the bundle remotely, with only a negligible load in the form of a particle. Using this technique, oscillation of live hair bundle was measured, which revealed that spontaneous oscillations were strongly affected, with an extension of the period and eventual suppression induced by a negative deflection of the bundle. This technique will be useful to many biological fields, as it can be applied to any system that detects mechanical signals, or is modulated by external mechanical strain. This project can provide some implication on the mechanical gating of its individual hair cell components.

II. Introduction

The acuity of auditory detection is largely the result of remarkable properties of hair cells - specialized cells of the inner ear that detect mechanical stimuli and transduce them into electrical signals.[1] These cells display extreme sensitivity – they can detect mechanical displacements smaller than 1 nm – and yet can withstand loud sounds, covering a range of about 6 orders of magnitude of applied pressure.[2] Hair cells can amplify incoming mechanical stimuli, sustain prolonged oscillations, and lead to the spontaneous emission of sound.[3] The biophysical mechanisms underlying this active amplification process have still not been fully explained, making the auditory and vestibular detectors among the least understood of the biological sensory systems. The understanding of the proper functioning and sensitivity of hair cells holds significance not only from the perspective of basic science but also for future generation highly sensitive nanoscale sensing device

developments as well.

Some important questions on this auditory sensory system are:

1) the force required to open an individual channel;

2) the way that calcium entry modulates the gating force; and

3) the development of a highly sensitive bionic sensory system which stimulates hair cell artificially.

Upon considering these questions, the overall goal of this project is to provide new method which can artificially control the mechanical oscillation and signaling process and mechanical properties in molecular basis. It is expected that this research will provide better understanding of hair cell signaling process and mechanical properties in molecular basis.

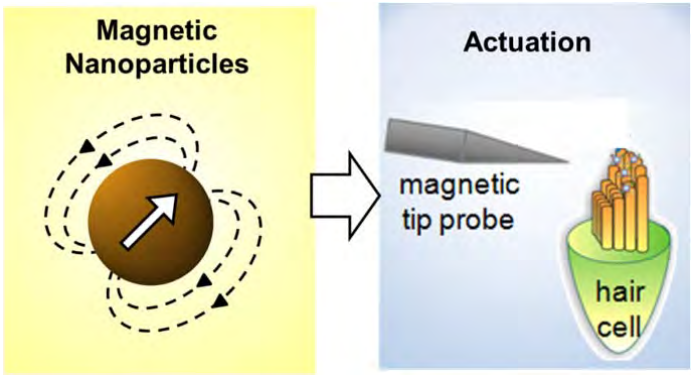

Figure 1. Magnetic nanoparticles as actuators for biological systems.

III. Approach:

In order to achieve this, the magnetic heterostructured nano-platform was designed to artificially manipulate the oscillation of hair cell and it was harnessed with enhanced magnetism, multifuctionality, and colloidal stability. In this approach superparamagnetic particle with approximately 1 micron size is used. This particle is iron oxide-based exhibiting relatively high magnetic susceptibility ($\chi_m = 720 \times 10^{-5}$) and saturation magnetization values (80 emu/gram), which provide adequate force for manipulating and imposing the deflection on hair cell bundles with a magnetic probe tip placed at varying distances from the particles. Based on this, the protein which specifically binds to the hair bundle was conjugated on the surface of the particle, providing the magnetic particle the targeting capability to the hair cells

(Figure 1). We used in vitro preparations of the bullfrog (*Rana catesbeiana*) sacculus, a planar preparation containing on the order of a thousand hair cells embedded in the supporting tissue. Then the magnetic probe tip was ramped close to the hair bundle and applied the controllable magnetic force to stimulate the motion of hair bundles. This bundle motion could be recorded by using a fast camera at a high resolution, and tracked with the software developed in MatLab.

The approach of magnetic particle enabled us to apply a force on the bundle remotely, with only a negligible load in the form of a particle. Spontaneous oscillations were strongly affected, with an extension of the period and eventual suppression induced by a negative deflection of the bundle (towards preferential closure of the channels).

IV. Experiment:

Magnetic particle preparation and conjugation to the hair bundle:

Superparamagnetic Fe_3O_4 particles (Thermo Scientific) of 1 μm diameter were modified with carboxyl groups and conjugated to Concanavalin-A, a lectin that binds to glycosides. 2-(N-morpholino)ethanesulfonic acid (MES, 25 mM final concentration), bead stock (1% solids final), Concanavalin-A (1 g/L final), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) (EDAC, 2.5 g/L final) were used in the reaction.[4]

Magnetic particle manipulation:

The above mentioned magnetic nanoparticles were firstly deposited onto a selective area of the cochlear preparation using a micropipette. Briefly, the nanoparticles were precisely sprayed to the specific region of the preparation on the microscope. Then, a magnetic tweezer system was applied to the hair bundle. A permanent magnet with sharp tapering was mounted onto a piezo-electric actuator and drawn near to the hair bundle.

Hair cell tissue preparation and microscopic observation:

We used in vitro preparations of the bullfrog (*Rana catesbeiana*) sacculus, a

planar preparation containing on the order of a thousand hair cells embedded in the supporting tissue. Hair bundles were imaged on an upright optical microscope (Olympus B51X) with a water-immersion objective, the images were further magnified to 760X and projected onto a high-speed Complementary Metal Oxide Semiconductor (CMOS) camera (Photon FASTCAM SA1.1). Bundle motion was recorded at (1024 x 1024)-pixel resolution at 1000 frames per second, and tracked with software developed in MatLab that extracts the center position of a bundle in each frame by fitting a Gaussian distribution to its intensity profile.[5]

Hair bundle tracking:

To determine the position of the hair bundle in each frame of view, we developed software that follows procedures established in the field of super-resolution imaging. A band-pass filter is applied to the first image of a record, and a 1-D fitting window is manually selected to match the width of a selected hair bundle. A Gaussian distribution is then fit to the unfiltered intensity profile. Time-dependent records of bundle displacement are obtained by plotting the center position extracted for each frame of the record.

V. Results and Discussion:

Force measurement of magnetic particles:

The force of single magnetic particle was measured by recording the flying movement of particles in the solution when the magnetic probe tip was drawn close to the particles. Photron fast camera was used for the recording of magnetic particles at 500 frames per second with the pixel spatial resolution of 50 nm. The movement of particle in each frame was analyzed to calculate the velocity of particle at different distances from the magnetic probe tip. Then the force of particles (F_d) was calculated by using the Navier-Stokes law. The results of 50 particle trackings were shown in Figure 2 where the plot is shown as the force of particle vs. the distance from the magnetic probe tip. The averaged force of particle at the 10 μm distance from the magnetic probe tip is ~11 pN. This value exceeds the threshold limit of bundle deflection and the gating spring of ion channel in the bundle.

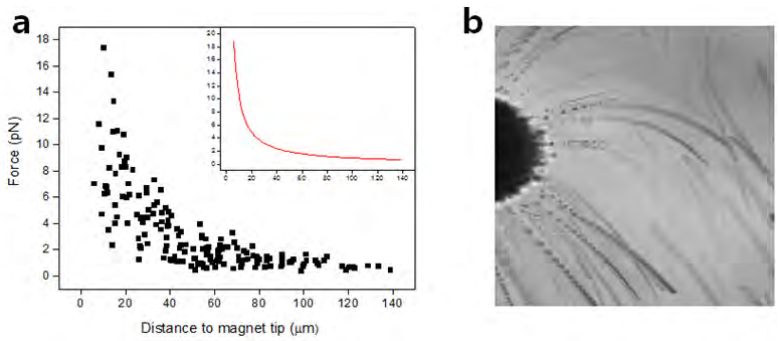

Figure 2. Force measurement of magnetic particles. (a) Plot of magnetic attraction force of particle vs the distance from the magnetic probe tip in aqueous solution. (b) Time lapse image of flying bead in the solution.

Magnetic manipulation of hair bundle:

The hair bundles were incubated with magnetic particles and a digital micromanipulator was used to apply a slow ramp to the position of the magnetic probe, bringing it into closer proximity to a selected bundle and thus increasing the magnetic force exerted on the Fe_3O_4 particles (Figure 3a). Figure 3b shows the effects of mechanical deflection imposed on hair bundles via magnetic actuation. Broken traces in the figure show the slow component of the bundle movement induced by the magnetic actuation. Spontaneous oscillations (shown in black) were strongly affected, showing a drastic reduction of the original frequency with increasing deflection, and were eventually fully suppressed by the imposed offset. Some variation was observed among the cells, with many also displaying a reduction in the amplitude of innate oscillation as the system transitioned into quiescence. Examples are shown from two different cells, with ramps applied in positive and negative directions.

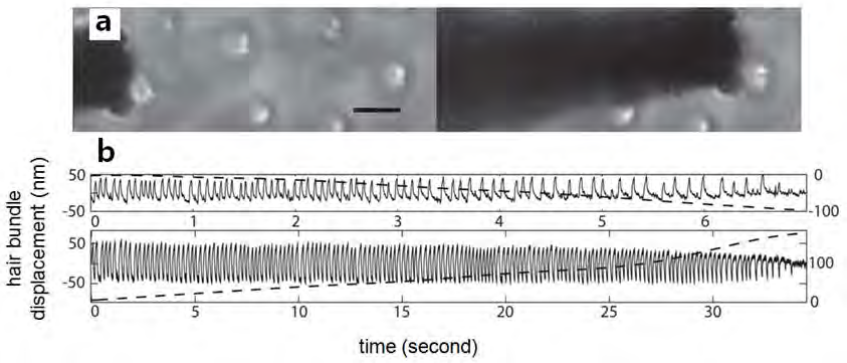

Figure 3. (a) First and last frame of a record during which a ramp was applied over 30 seconds to the position of the magnetic probe. Scale bar denotes 5 μm. (b) Traces of hair bundle motion obtained from two different cells under slow displacement ramps. The dashed line indicates the slow component of bundle motion, low-pass filtered at 0.5 Hz; the scales are given on the right vertical axes of the graphs. Solid black lines show bundle oscillations with the slow component subtracted

We directly observed the transition from multi-mode to single-mode oscillation and from the oscillatory to the quiescent state, under effectively load-free conditions. To access this regime, we introduced a new technique of mechanical actuation, attaching paramagnetic particles to the hair bundle and manipulating them via a permanent-magnet probe. Magnetic manipulation provides a potentially very versatile tool for probing cell dynamics, as it avoids many adverse effects of mechanical or optical techniques - it readily penetrates bio- logical tissue and does not heat or otherwise damage the cells. We applied the technique to demonstrate that external mechanical manipulation of the resting position of a hair bundle induces a crossover from multi- to single-mode oscillation, and eventually suppresses the innate motility. Our results indicate that offset deflection therefore constitutes a potential control parameter that determines the dynamic state of a hair cell.

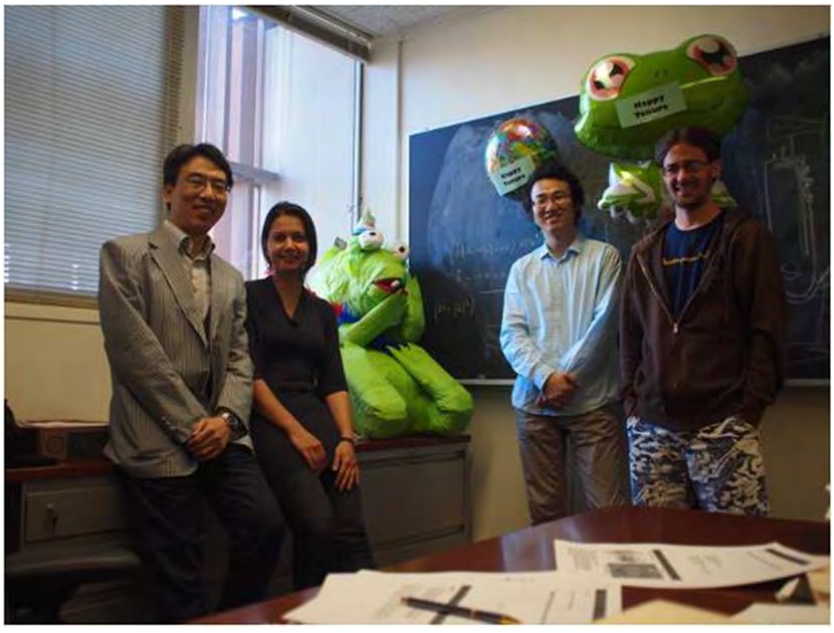

Figure 5. This work was resulted from the collaborational research with Prof. D. Bozovic,

UCLA. Left to right: Prof. J. Cheon, Prof. D. Bozovic, J.-H. Lee, and D. Rowland.

VI. References

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VII. List of Publications:

D. Rowland, Y. Roongthumskul, J.-H. Lee, J. Cheon, D. Bozovic, Magnetic Actuation of Hair Cells. *Appl. Phys. Lett.* **2011**, in preparation.

VIII. Financial Reports:

EXPENDITURES (Jul. 1, 2010~ June. 30, 2011)	
SALARIES	$4,800
OTHER DIRECT COSTS	
C. MATERIALS AND SUPPLY	$10,000
purchasing reagents and microscope set-up	
D. TRAVEL	$15,900
Collaboration visits	
Conferences	
E. TELECOMMUNICATION FEE	$800
F. TOTAL DIRECT EXPENSES	$31,500
G. ADMINISTRATION EXPENSES	$3,500
H.TOTAL	**$35,000**

***Appendix**

Paper in preparation:

Magnetic actuation of Hair Cells

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(Dated: 5 August 2011)

The amphibian sacculus contains mechanically sensitive hair cells whose stereociliary bundles oscillate spontaneously when decoupled from their overlying membrane. Previous studies have shown that a steady-state offset on the resting position of an individual hair bundle can serve as a control parameter that can suppress or modulate the native oscillation. To probe the innate dynamics of the spontaneous oscillation in the proximity of the critical point, we describe here a method for mechanical actuation that avoids loading the bundles or contributing to the viscous drag. Mechanical stimulation was accomplished with a permanent magnetic probe controlled with a piezoelectric actuator and 1 μm paramagnetic iron-oxide beads attached to the tips of the stereocilia. Deflections were ramped at various speeds and shown to suppress hair bundle oscillations. The temporal profiles were likewise significantly affected, showing transitions from multi-mode to single-mode oscillation under the imposed offset. Our results provide evidence that resting-state position of the hair bundle constitutes a potential control parameter that fine-tunes the internal dynamics of the cell.

PACS numbers: 43.64.Yp
Keywords: Magnetic actuation, bullfrog, hair cells, oscillation

The auditory system can detect induced deflections as small as a few Å, while being able to withstand applied pressures spanning orders of magnitude[1]. *In vivo* measurements of basilar membrane motion have revealed strong nonlinearities in the response of the system[2], shown to be crucial to the sensitivity of detection and the compression of the dynamic range. Mechanical sensing is performed by hair cells, named in reference to the bundles of stereocilia protruding from the cells' somae (Fig. 1a). Vibrations evoked by sound waves deflect the hair bundles and stretch the filamentous connections between the stereocilia, directly gating the opening of mechanically sensitive ion channels. Hair cells are immersed in an aqueous environment, and thus require an energy-consuming process to maintain oscillation against viscous dissipation. They were shown to actively amplify imposed stimuli in a frequency-selective manner, with the gain and sharpness of tuning decreasing with increasing stimulus levels[3]. The presence of an active process in auditory detection was first proposed in 1948[4], but its biophysical mechanisms have still not been fully resolved.

Theoretical models have shown that the compressive response by inner ear hair cells can be described by nonlinear differential equations containing a cubic term[5–7]. They predict the existence of a bifurcation separating the regime where the system can amplify but is quiescent in the absence of input, and one which gives rise to a stable limit-cycle oscillation. A control parameter tunes the system towards or away from the bifurcation and thus adjusts the sensitivity of response. Potential biological manifestations of this bifurcation-crossing include the phenomenon of spontaneous otoacoustic emission[8] observed *in vivo* across many species, and spontaneous limit-cycle oscillations exhibited by hair bundles *in vitro*[9,10].

Spontaneous oscillations arise from an interplay between two processes: mechano-electrical transduction that causes the ion channels to flicker between open and closed states, and a slow adaptation process that adjusts the position of the channels along the length of a stereocilium to maintain optimal tension in the tip links[11]. We demonstrated previously that steady-state offsets imposed on the resting position of the stereociliary bundle constitute a potential control parameter that can induce a transition from the oscillatory to the quiescent state[12]. Rich dynamics were observed near the critical point, with characteristics of an infinite-period bifurcation, a supercritical Hopf bifurcation[13], or an admixture of the two.

Mechanical stimulation of hair bundles has previously been delivered with jet fluids or with elastic glass rods attached to the tips of the stereocilia, which inevitably imposes elastic or viscous loading on the system. As we have seen that loading strongly affects hair cell dynamics and qualitatively changes the temporal profile of spontaneous oscillations[14], we pursued a method that eludes this external load and then allows us to probe the innate dynamics of freely oscillating bundles. We here present on the use of a novel technique for mechanically actuating the stereocilia. We attach paramagnetic micro-particles to the stereocilia, and manipulate them with a magnetic probe placed at varying distance form the particles to impose deflections on the hair bundle.

We used *in vitro* preparations of the bullfrog (*Rana catesbeiana*) sacculus, a planar preparation containing on the order of a thousand hair cells embedded in the supporting tissue. The epithelia were exposed to different

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